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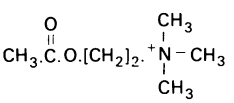
THE DISCOVERY OF BRETYLIUM AND BETHANIDINE

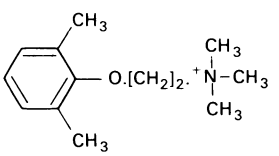
A.F. GREEN
The Wellcome Research Laboratories,
Langley Court, Beckenham, Kent

Serendipidy was a major factor in the discovery of the forerunner of bretylium. Starting in the early 1950's, Dr Hey of Leeds University had been examining the relationship between structure and nicotine-like action in a series of substituted phenylcholine ethers (Hey, 1952). As an extension of this work choline 2:6 xylyl ether bromide (Figure 1) was made and examined (Hey & Willey, 1954). This compound became known as TM10 and later as xylocholine. It produced a brief rise in blood pressure in cats, characteristic of the series, but tachyphylaxis to this response was unusually rapid in onset and falsely attributed to a long lasting local anaesthetic action. Later the important observation was made that TM10 abolished, for long periods, contractions of the nictitating membrane caused by stimulation of the postganglionic cervical sympathetic nerve, without impairing, except briefly, the contractile responses of the membranes to injected adrenaline. These studies were extended by Exley (1957) who demonstrated that TM10 also blocked the effects of stimulating the sympathetic nerves to the heart, uterus, salivary glands and spleen, but not the corresponding effects on these tissues of injected adrenaline and noradrenaline; the action was generalised. Moreover he confirmed directly that TM10 suppressed the release of the adrenergic transmitter from the splenic nerve. Unfortunately, this compound was not suitable for clinical study because of prominent cholinomimetic properties. For example, in cats, doses that were sufficient to relax the nictitating membranes, i.e. to impair sympathetic nerve function, also caused salivation and lachrymation. If a compound could be found that only affected adrenergic nerve function it would be expected to offer major advantages over the ganglion blocking agents, then the most effective drugs for the treatment of hypertension. The patients would be spared the multiple troublesome effects associated with blockade of transmission in parasympathetic ganglia that inevitably attended the wanted blood pressure lowering action produced by blockade of sympathetic ganglia.

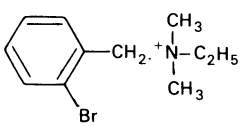
Acetylcholine



Xylocholine (TM10)



Bretylium



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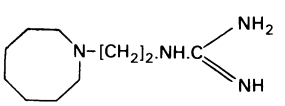

Bethanidine

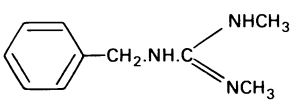

Figure 1 Bretylium and related compounds.

A search for a compound with the desired properties was initiated at the Wellcome Research Laboratories UK in 1956 where we had gained from the experience of Professor Bain and his colleagues at Leeds University, and had relevant expertise arising from our development of long-acting ganglion blocking agents (Green, 1956). Furthermore, we had a background of relevant chemistry derived from the development of anthelminthics such as bephenium (Copp *et al.*, 1958) with structures somewhat similar to that of TM10. Bephenium itself had weak sympathetic blocking properties but BW25C57 was much nearer to having the desired properties, except that its sympathomimetic properties seemed too prominent. By the end of 1957 Alan Boura and I had selected bretylium (BW373C57) as a candidate for developing for clinical trial. Its effects on adrenergic mechanisms closely resembled those of TM10 but its cholinomimetic effects were minimal. In the first publication

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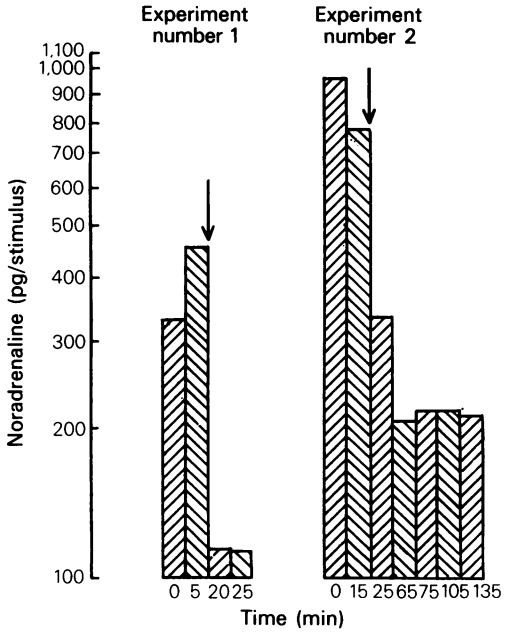

Figure 2 The effect of bretylium on the noradrenaline content of the venous blood from the spleen during stimulation of the splenic nerve. Stimuli were applied to the nerve at the rate of 25/s for 10 s in Experiment number 1 and for 60 s in Experiment number 2. The venous blood was collected for 30 s from the beginning of stimulation in Experiment number 1 and for 2 min in Experiment number 2. The output of noradrenaline, assayed in the pithed rat, is expressed in pg/stimulus. The arrows indicate the intravenous injection of 10 mg/kg of bretylium in Experiment number 1 and 5 mg/kg in Experiment number 2. (From Boura & Green, 1959).

on this compound (Boura *et al.*, 1959a) it was referred to as an 'antiadrenergic', on the advice of Sir Henry Dale who rued that the term 'adrenergic blocking' was already being misused for adrenaline antagonists. However, the Editors of the British Journal of Pharmacology preferred the less ambiguous expression 'adrenergic neurone blocking agent' which has come to be generally accepted.

The prime feature of these compounds is that they inhibit the release of the adrenergic transmitter. This can readily be shown using the splenic nerve preparation developed by Sir Lindor Brown (Figure 2) or by a variant of it in which [^{3}H]-noradrenaline is first introduced into the spleen so that measurement of released transmitter is facilitated. The greater sensitivity of the latter method allows it to demonstrate directly the release of noradrenaline from the spleen by bretylium. Such a displacing effect on neuronal noradrenaline is a second characteristic of all known adrenergic neurone blocking agents. It accounts for the sympathomimetic manifestations in experimental animals and in man, especially when the dosage is high and the drug is administered intravenously as a bolus.

A third characteristic property of adrenergic neurone blockade was emerging before the first clinical study of bretylium. This is the enhancement of tissue sensitivity to adrenaline and more especially to noradrenaline, resembling that following surgical postganglionic sympathectomy. The first observations of this were in acute studies (Boura & Green, 1959). Later, it was found during the daily administration of bretylium to cats that the extent of the resulting relaxation of the nictitating membrane produced became progressively less and that this was explained by growing sensitivity to the adrenergic transmitter (Green, 1960; Boura & Green, 1962).

In 1959 a summary of the available pharmacological information was published together with the observations on bretylium tosylate (Darenthin) made in volunteers and hypertensive subjects in Professor Rosenheim's department at University College London (Boura *et al.*, 1959b). The findings in man were essentially those predicted from animal studies. First, evidence was obtained of sympathetic block in healthy volunteers by intravenous injection of bretylium; the lesson was quickly learnt that, if such expressions of sympathomimesis as palpitations and a sensation of retrosternal oppression were to be minimised, it was advantageous to replace bolus intravenous injection by slower administration. Supine blood pressure was not affected but postural hypotension was prominent in most subjects given intravenous doses of 0.5 to 1 mg/kg, in keeping with

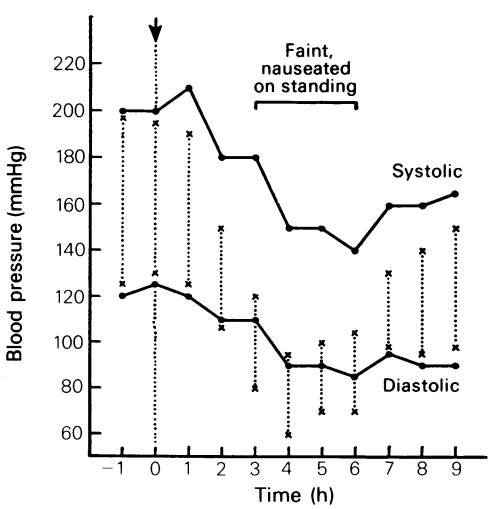

Figure 3 Effect of a single oral dose of bretylium 328 mg (500 mg of the iodide) on the blood pressure of a hypertensive subject (supine ●, standing ×). (From Boura *et al.*, 1959b).

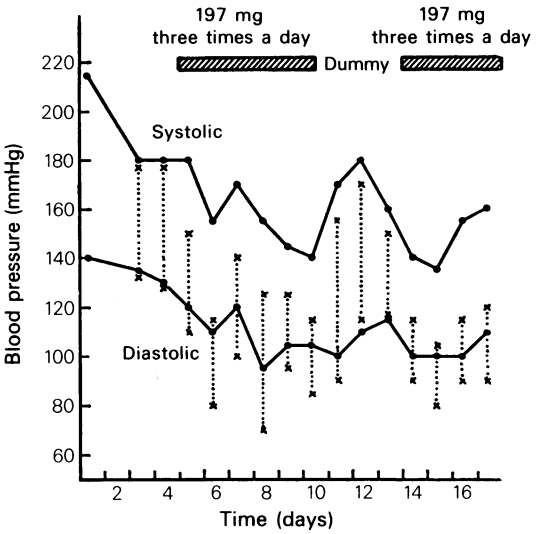

Figure 4 Effect of regular oral doses of bretylium tosylate on the blood pressure of a hypertensive subject (supine ●, standing ×). (From Boura *et al.*, 1959b).

previous observations using cats. Measurements of the circulatory response to Valsalva's manoeuvre by Professor A.C. Dornhorst in a volunteer confirmed sympathetic blockade. Intraarterial infusions of 7.5 mg bretylium caused partial sympathetic block, a sympathomimetic vasoconstrictor response preceding a vasodilator response as in animals. Postural hypotension was also observed in some but not all normal subjects who received oral doses in the range of 150–328 mg. There were no serious side effects. On the basis of this information in normal subjects, bretylium was injected intravenously in three hypertensive volunteers. Single oral doses of 197–328 mg bretylium were then given to four hypertensive subjects and the effects in the one showing the greatest changes are shown in Figure 3. Regular oral treatment with bretylium was then started in hypertensive subjects; Figure 4 represents a typical response, but blood pressure was not effectively controlled in some subjects. A patient with malignant hypertension responded well to a combination of bretylium and chlorothiazide. None of these subjects displayed any serious unwanted effects and the great advantage of bretylium in not causing the troublesome impairments of parasympathetic function was much acclaimed, especially by patients who previously had been subjected to ganglion blocking agents.

In these early studies in patients treated for up to 6 months, it was accepted that some tolerance probably did occur but this had not presented a serious problem. As concluded at the time, the practice of starting with a small dose of the drug and gradually increasing the dose one to three times a week would mask minor degrees of tolerance.

During 1960 experience in the effects of bretylium in man grew rapidly both with respect to its actions on adrenergic mechanisms and its clinical value in hypertension (Blair *et al.*, 1960; Campbell & Montuschi, 1960; Conway, 1960; Dollery *et al.*, 1960b; Freis *et al.*, 1960; Hayden & Boake, 1960; Hodge & Smirk, 1960; Laurence & Rosenheim, 1960; Smirk, 1960; Taylor & Donald, 1960). In general the results were in keeping with those in the first study of bretylium in man but there was increasing concern that whereas the patients were spared the crippling effects of parasympathetic block, bretylium produced several undesirable effects. These included dizziness, increased frequency of micturition, muscle weakness and parotid pain. As summed up by Professor Turner in 1960 'Side effects are numerous although relatively mild and most could be tolerated if bretylium was a more effective therapeutic agent'. Regarding effectiveness, the major problems were the progressive development of tolerance that could not always be overcome by readily ingestable amounts of drug, the insensitivity of many patients even to large doses and the variability of response associated with the irregular absorption of a quaternary ammonium salt. Guanethidine became available in the UK in 1960 and superseded bretylium essentially because of its greater effectiveness and reliability, a smaller dose and a lesser liability to cause insuperable tolerance; its side effects were just as common, more prolonged, and in some respects more intense.

In 1960 it was questionable from the pharmacological properties described for guanethidine (Bein, 1960; Maxwell *et al.*, 1959; Maxwell *et al.*, 1960) whether it had the same mechanism of action as bretylium. The route of discovery of guanethidine was entirely separate and the pharmacological methods used by Dr R.A. Maxwell and his colleagues were different. However, the similarities between the two compounds became increasingly clear as pharmacological investigations proceeded (for reviews Green 1962; Boura & Green, 1965; Maxwell & Wastila, 1977; Armstrong & Green, 1980). However, two major distinctions remain, both of which we took into consideration at the Wellcome Laboratories when in the early 1960s we sought a better adrenergic neurone blocking agent than either bretylium or guanethidine. The first distinction is that guanethidine, in contrast to bretylium, causes a fairly rapid depletion of tissue noradrenaline. At first some thought this to be the primary cause of neurone blockade by guanethidine, as is the case for reserpine, but Cass & Spriggs (1961) showed that adrenergic neurone blockade preceeds the loss of noradrenaline. Adrenergic neurone blockade ensued rapidly following subcutaneous injection of guanethidine but tissue

noradrenaline content fell substantially only after about 4 h. Nevertheless such loss can sustain neuronal block by guanethidine and, as it seemed desirable to have a compound with a shorter duration of action, we aimed at finding a compound that caused less noradrenaline depletion.

The second distinction concerns effects on curves relating the frequency of sympathetic nerve stimulation to the end organ response. In studies of such curves for the nictitating membrane response in cats the effect of increasing doses of bretylium was to progressively depress the slope of the curve (Boura & Green, 1959). Responses to low rates of nerve stimulation were minimally affected. Moreover, when during daily administration of bretylium marked hypersensitivity to the adrenergic transmitter had developed, situations arose where the responses to low rates of nerve stimulation had become greater than in control animals (Green, 1960; Boura & Green, 1962). In contrast the effect of guanethidine was to shift the curve to the right, with preferential suppression of the effects of low rates of nerve stimulation. Enhanced sensitivity of the membrane to the adrenergic transmitter develops during continued guanethidine treatment and tends to push the curve to the left, but the cumulative effects of guanethidine have an opposing action. The curve remains parallel to that in controls and exaggerated responses to low rates of stimulation do not occur. The maximum sensitisation to noradrenaline that develops during daily administration of either drug is the same and is equal to that following postganglionic nerve section, —in the case of the cat nictitating membranes this can be as much as 100-fold (Boura & Green, 1962). Hence, it is the differing effects of the two drugs on nerve frequency response curves (Figure 5) that provides the most plausible explanation of why tolerance was a greater problem in the clinical use of bretylium than of guanethidine. These differing effects on frequency response curves apply also to several other end organ responses to adrenergic nerve stimulation (Green & Robson, 1964; Boura, 1967) or cholinergic nerve stimulation (Green & Hughes, 1966). However, the degree of sensitisation to the adrenergic transmitter produced by adrenergic neurone blockade, as indicated by tissue sensitivity to injected noradrenaline, varies considerably between different tissues and is often much less than that found in cat nictitating membranes (Boura & Green, 1962; Green & Robson, 1965; Boura, 1967; Armstrong & Boura, 1970; Maxwell & Wastila, 1977). Hypersensitivity to injected noradrenaline occurs with both drugs in man, but a systematic study of its progression during treatment seems not to have been made (Laurence & Rosenheim, 1960).

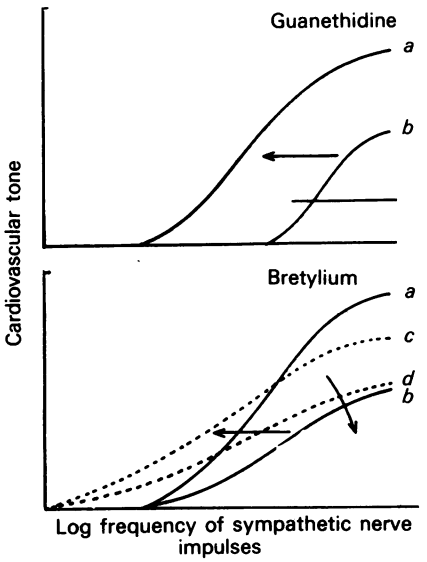

Figure 5 Theoretical curves illustrating a possible explanation for the finding that the incidence of tolerance to adrenergic neurone blockade may be higher with bretylium than with guanethidine.
Guanethidine: The curve relating frequency of stimulation to effect produced in untreated animals (a) shifts to the right after giving the drug (b). The tendency for the curve (b) to shift to the left during the development of hypersensitivity to the adrenergic nerve transmitter that accompanies daily administration of the drug is apparently offset by the cumulative effect of the drug or can be overcome by increasing the dosage.
Bretylium: The slope of the curve (a) is depressed after giving bretylium (b) to an extent depending on dosage. When bretylium has been given daily the developed hypersensitivity to adrenergic transmitter may be expected to cause a parallel shift of the curve (b) to a position (c), so that responses to low rates of stimulation tend to exceed those before treatment (curve a). Increased dosage of bretylium is expected to depress the slope of the curve (c) to position (d), but, except when the dosage is large, responses to the lowest rates of stimulation may continue to exceed those before treatment. (From Boura & Green, 1962.)

The nature of effect on frequency response curves is also relevant to the comparative hypotensive effects of the two drugs that depend on whether a subject is supine, standing or exercising (Boura & Green, 1962). From a consideration of Figure 6 it would be expected that when the dosage of each drug is chosen as that which causes the desired lowering of cardiovascular tone with the subject standing, then guanethidine would cause the greater lowering of blood pressure when the subject was supine and bretylium when the subject was exercising. Also because it more completely blocks low rates of sympathetic traffic, guanethidine would be expected to produce more bradycardia than bretylium. These expectations were entirely in keeping both with clinical observations then available, such as those of Taylor & Donald

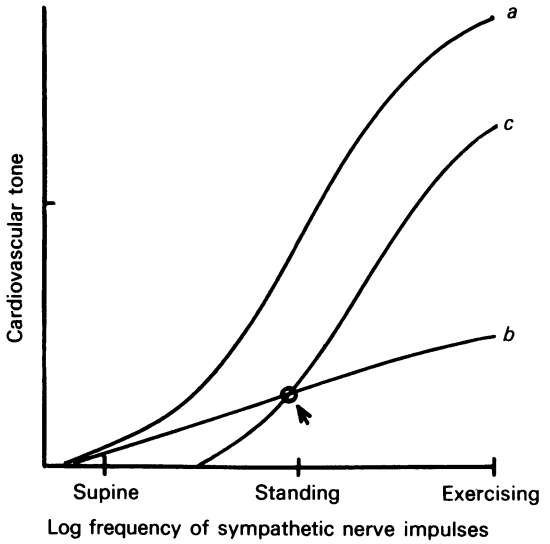

Figure 6 Expected relative effects of bretylium and guanethidine on cardiovascular tone in man depending on whether a subject is supine, standing or exercising. It is assumed that the frequency of sympathetic nerve impulses is least when the subject is supine and greatest during exercise and that the relation of this frequency to cardiovascular tone (control = a) is affected by bretylium (b) and guanethidine (c) in like manner to the nerve frequency-nictitating membrane response curves. The dose of bretylium or guanethidine usually gives is that which causes the desired lowering of cardiovascular tone with the subject standing—this is represented by the circle at the intersection of the curves. (From Boura & Green, 1962.)

(1960) and Dollery *et al.* (1960a) and with later studies, thus providing a rational explanation of them.

The important criteria for an improved adrenergic neurone blocking agent seemed therefore to be minimal depletion of neuronal noradrenaline (i.e. bretylium-like), a parallel shift of the nerve frequency-end organ response curve (i.e. guanethidine-like) and complete absorption from the alimentary tract (≥ guanethidine). Another criterion was the avoidance of the severe diarrhoea associated with guanethidine but not with bretylium.

Of the many compounds examined by us in animals, the one that appeared most likely to satisfy the above criteria was BW467C60 later known as bethanidine (Boura *et al.*, 1961a; Boura *et al.*, 1962; Boura & Green, 1963). The fit with the criteria was not exact. Its effects on frequency response curves are intermediate between those of bretylium and guanethidine as for example in Figures 7 and 8 where the curves are shifted to the right but with some depression of slope. Likewise its effects on neuronal stores of noradrenaline are intermediate (Costa *et al.*, 1962; Boura & Green, 1963, 1965). The chemical similarities of bethanidine to both bretylium and guanethidine are obvious from Figure 1 but bethanidine is in fact the 85th of some 290 guanidine derivatives that we examined. (Bretylium was the 121st of some 450 quaternary ammonium compounds examined.) Bethanidine and its ortho-chloro derivative, which latter resembled bretylium in its effects on nerve frequency response curves and on neuronal noradrenaline stores (Costa *et al.*, 1962; Boura & Green, 1963), were first studied in hypertensive subjects by Montuschi & Pickens (1962). Bethanidine itself was the preferred compound. Other clinical reports that followed soon after were also generally favourable (Johnston *et al.*, 1962; Smirk, 1962, 1963). The only clinical aspects on which comment will be made here relate to the effects on frequency-response curves and on neuronal noradrenaline stores in animals being intermediate between those of bretylium and guanethidine. Regarding tolerance, the expectation would be that dosage would more often need upward adjustment during early treatment than in the case of guanethidine but that runaway tolerance would not be encountered. This was the case. Rather greater postural hypotension than with guanethidine was expected and was found (Prichard *et al.*, 1968; Prichard & Walden, 1979). Lesser depletion of neuronal noradrenaline than with guanethidine leads to the expectation of earlier restoration of neuronal function when treatment is withdrawn and this is common experience for example in the context of the return of ejaculatory function in man.

The physiology and biochemistry of adrenergic mechanisms was ill-understood at the time of the discovery of the blocking agents. There should therefore be no surprise that many of the early clues relating to the probable mode of action of the blocking agents have been misinterpreted and have led to false conjectures. Mention will be made here only of the major clues and their current interpretation presented in reviews (Boura & Green, 1965; Maxwell & Wastila, 1977; Armstrong & Green, 1980; Boura & Green, 1981). First is the clue dating from 1954 that TM10 has a powerful local anaesthetic action of unusually long persistence (Hey & Willey, 1954). This action is shared by bretylium (Boura & Green, 1959) and many other adrenergic neurone blocking agents (Boura & Green, 1965). Second is the clue that emerged in 1960. In cats [^{14}C]-bretylium is avidly taken up into adrenergic nerves against a steep concentration gradient to reach surprisingly high concentrations in these but not in cholinergic nerves (Figure 9; Boura *et al.*, 1960b). Furthermore adrenergic nerves similarly concentrate BW172C58 the powerfully blocking benzoyl derivative of TM10 (Boura *et al.*, 1960a; Boura *et al.*, 1961b), bethanidine (Boura *et*

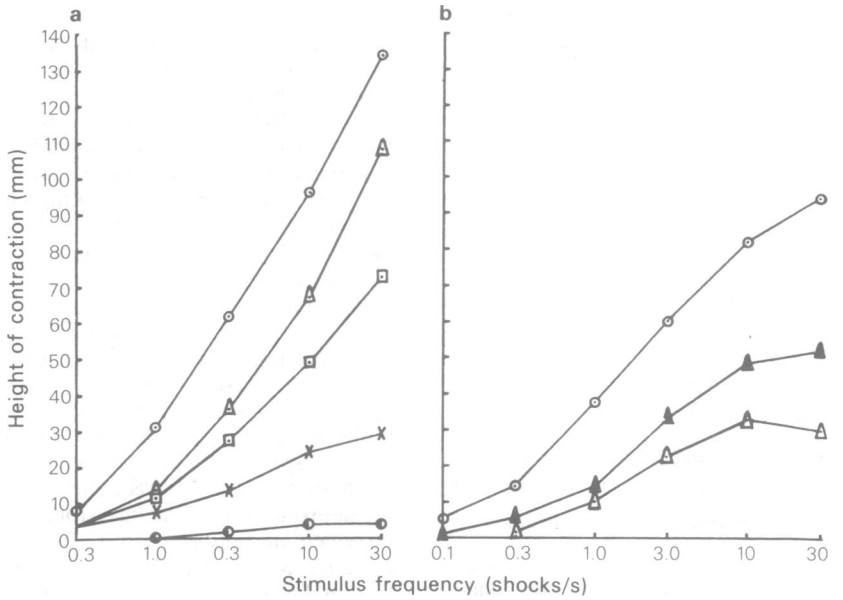

Figure 7 Mean heights of contraction of the nictitating membranes of cats caused by stimulation of the pre- or post-ganglionic cervical sympathetic nerve at various stimulus frequencies for 1 min periods. (a) effects of intravenous BW 467C60 in an acute experiment using chloralose as the anaesthetic agent and post-ganglionic stimulation: O——O initial; Δ——Δ after 0.1 mg/kg; □——□ after 0.3 mg/kg; ×——× after 1 mg/kg; ◐——◐ after 3 mg/kg of BW 467C60. (b) responses to pre-ganglionic stimulation 24 h after subcutaneous injection of various doses of BW 467C60 into cats anaesthetized with pentobarbitone: O——O controls (eleven cats); ▲——▲ after 0.5 mg/kg (three cats); Δ——Δ after 1 mg/kg of BW 467C60 (three cats). There was no response of the membranes 24 h after 3 mg/kg of BW 467C60 (two cats). (From Boura & Green, 1963).

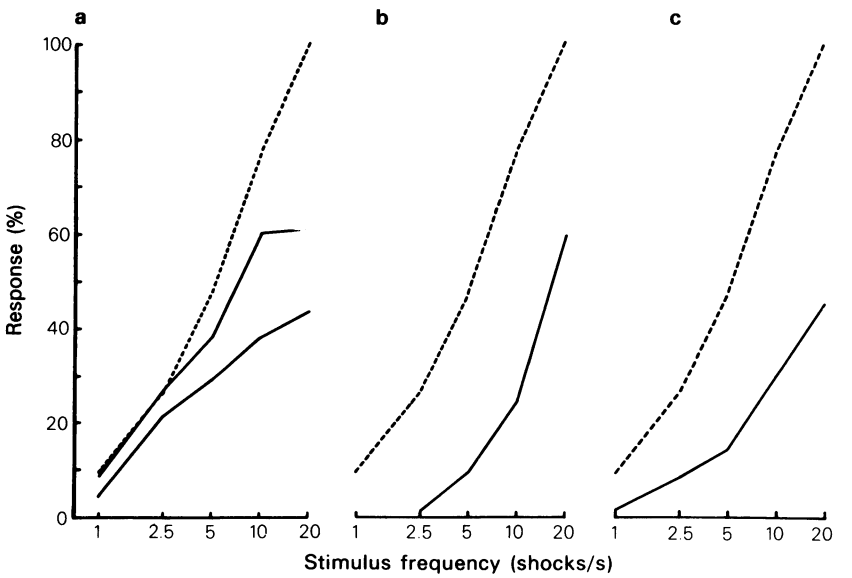

Figure 8 The relationship of the frequency of splenic nerve stimulation (on log scale) to changes in spleen volume in anaesthetized cats. Each train of stimuli was applied for 30 s and adequate time was allowed for the spleen to relax fully between each train. All spleen volume changes have been expressed as a percentage of that produced by 20 shocks/s in each animal before giving blocking agents. - - - control responses (mean for twelve cats). (a) after 1.0 and approximately 2.5 mg/kg of bretylium tosylate respectively, showing depression of slope; (b) after 0.3 mg/kg of guanethidine sulphate showing a parallel shift of the regression line; (c) after 0.3 mg/kg of bethanidine hydrochloride which produced an effect on the regression line intermediate between those of bretylium and guanethidine. Experimental means are for groups of three cats. (From Green & Robson, 1964.)

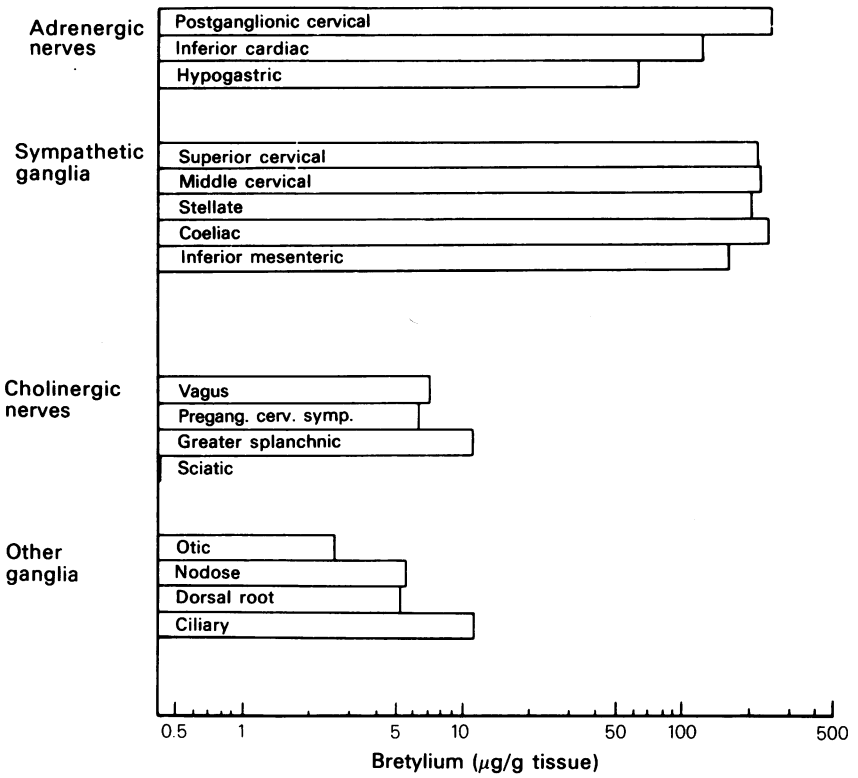

Figure 9 The concentration of [^{14}C]-labelled bretylium in the peripheral nerve tissue of cats, at 12–18 h after 10 mg/kg of labelled bretylium iodide subcutaneously. Bretylium: molecular weight = 243; 100 μg = 412 mμ moles. (From findings of Boura *et al.*, 1960b as presented by Green, 1962.)

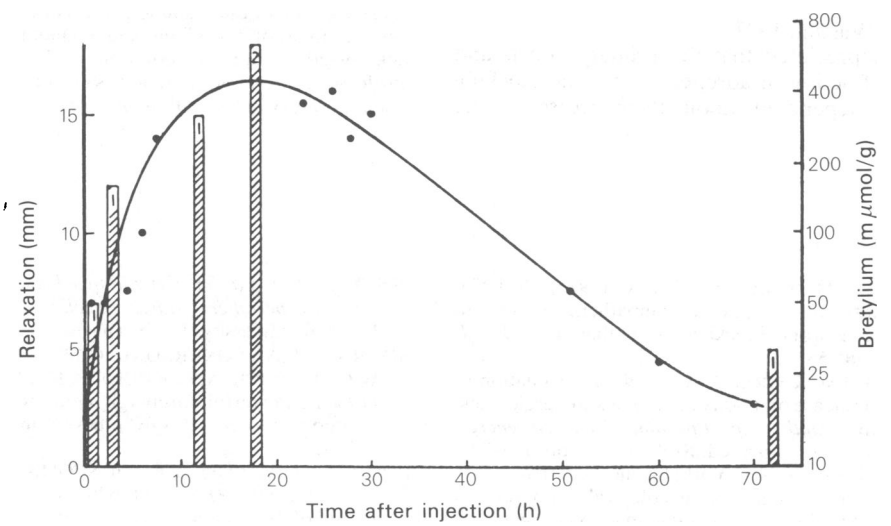

Figure 10 Relaxation of the nictitating membranes of cats, related to the concentration of bretylium in sympathetic ganglia. The curve shows the exposure of the membrane at intervals after 10 mg/kg bretylium s.c. (from Boura & Green, 1959; means for 4 cats, measuring along the lower lid). The columns represent the mean concentration of the drug in the superior cervical, stellate and coeliac ganglia, expressed in mμ moles/g tissue, after 10 mg/kg. [^{14}C]-labelled bretylium iodide s.c.; at each time interval 1 or 2 cats were used. (From Boura *et al.*, 1960, after Green, 1960).

al., 1962), and guanethidine (Chang *et al.*, 1964, 1965; Mitchell & Oates, 1970). Moreover the high concentration of bretylium that accumulate in adrenergic nerves are temporally related to impairment of adrenergic function (Boura *et al.*, 1960b; Green, 1960, Figure 10) and are at least coincident with it in the case of the other above mentioned blocking agents. These concentrations of adrenergic neurone blocking agents are sufficient to impair conduction, in only some isolated nerve trunks but they are fully adequate to completely block a variety of end organ responses to adrenergic nerve stimulation in isolated preparations. Some responses to cholinergic nerve stimulation are also blocked in isolated preparations but, as already said, adrenergic neurone blocking agents do not concentrate in cholinergic nerves in intact animals. Next came abundant evidence that the mechanism whereby the adrenergic neurone blocking agents are concentrated in adrenergic neurones is indeed the same mechanism as that whereby noradrenaline is normally retaken into the neurones to terminate its transmitter function, and named Uptake 1 by Iverson (1971) (for review see Maxwell & Wastila, 1977). Yet further evidence that adrenergic neurone blocking agents act by depressing the excitability of adrenergic nerve terminals into which they are concentrated by the Uptake 1 process, even though they do not, at reasonable dosage, impair conduction in adrenergic nerve trunks (Exley, 1960), has been provided. Bretylium and guanethidine suppress antidromal discharges in cardiac and splenic nerves elicited by acetylcholine and potassium chloride (Haeusler *et al.*, 1968; Haeusler *et al.*, 1969; Maxwell & Wastila, 1977).

Having appreciated that the primary effects and specificity of action of adrenergic neurone blocking agents are dependent upon their access to the neurone by the Uptake 1 process, appreciation follows of their interaction with other drugs that are known to compete for this process. Such drugs include cocaine, dexampethamine and the tricyclic antidepressants all of which are well known to antagonise adrenergic neurone blockade (Day, 1962; Day & Rand, 1963; Boura & Green, 1965; Maxwell & Wastila, 1977). Their inhibitory effect on the monoamine oxidase within adrenergic neurones seems to explain why some adrenergic neurone blocking agents conserve neuronal noradrenaline in some situations (Kuntzman & Jacobson, 1963; Boura & Green, 1965; Giachetti & Shore, 1967; Maxwell & Wastila, 1977).

The advent of the adrenergic neurone blocking agents was heralded by physicians and patients as it provided a treatment of hypertension that was not accompanied by the crippling side effects of the ganglion blocking agents. Their very nature of action however must necessarily result in the magnitude of their lowering of blood pressure being dependent on posture and exertion. Such dependance does not occur with the newer types of drug such as the β-adrenoceptor blockers and when they are effective they are clearly preferred. Nevertheless the adrenergic neurone blocking agents continue to have an important place in therapy and their discovery and study has provided a major contribution to the understanding of the physiology of adrenergic mechanisms.

The author is appreciative of the valuable contributions of Sir John McMichael, Professor C.T. Dollery and the Hypertensive Clinic of the Hammersmith Hospital to the evaluation in man of bretylium and guanethidine and gladly acknowledges the collaboration of Dr F.C. Copp and Professor A.L.A. Boura that was essential to the discovery and laboratory investigation of these drugs.

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